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Variability in seed quality traits in castor germplasm

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SUMMARY: Castor is an industrial oilseed crop with great potential for biorefineries. However, little is known about the variability in the bioactive compounds in castor germplasm. This study evaluated seed weight, oil content, fatty acid profile, tocopherols, and phytosterols in 160 accessions of the USDA-ARS castor germplasm collection. The accessions were grown in Cordoba, Spain, under three different environmental conditions. Environmental and genotype-by-environment interaction effects were predominant for most traits, resulting in moderate to low broad-sense heritabilities, which ranged from 0.12 for total tocopherol content to 0.88 for hundred-seed weight. The genetic variability in the seed quality traits identified in the collection was lower than that reported previously for the germplasm of wild and semi-wild accessions from Spain, which is attributed to the lower genetic diversity in cultivated than in wild forms. The variation in seed quality traits in castor germplasm can be exploited to improve the concentration of bioactive compounds in castor cultivars.

KEYWORDS: *Fatty acids; Oil content; Phytosterols; Ricinus communis; Seed weight; Tocopherols.*

RESUMEN: ***Variabilidad de los parámetros de calidad de las semillas en germoplasma de ricino*****.** El ricino es un cultivo industrial con gran potencial para biorrefinerías. Sin embargo, hay escasa información sobre variabilidad de compuestos bioactivos en germoplasma de ricino. El objetivo de este estudio fue la evaluación del peso de semilla, contenido en aceite, perfil de ácidos grasos y contenido de tocoferoles y fitoesteroles en germoplasma de ricino. Ciento sesenta entradas de ricino se cultivaron en Córdoba, España, en tres ambientes. Los efectos del ambiente y de la interacción genotipo por ambiente fueron predominantes para la mayoría de los caracteres, lo que resultó en moderada a baja heredabilidad, entre 0.12 para el contenido en tocoferoles a 0.88 para el peso de semilla. La variabilidad genética para caracteres de calidad en esta colección fue menor que la encontrada previamente en germoplasma de accesiones silvestres y ruderales, lo que se atribuye a la menor diversidad genética en las formas cultivadas. La variabilidad identificada en este estudio será de utilidad para aumentar la concentración de compuestos bioactivos en cultivares de ricino.

PALABRAS CLAVE: *Ácidos grasos; Contenido en aceite; Fitoesteroles; Peso de semilla; Ricinus communis; Tocoferoles*

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1. INTRODUCTION

On a world scale, castor (*Ricinus communis* L.) is a minor crop. It is cultivated on around 1.3 million ha, of which 0.9 million ha are in India (FAOSTAT, 2021). Castor seeds are a very rich source of oil, which can reach close to 60% of the whole seed weight (Fernández-Martínez and Velasco, 2012). The oil is used for biofuel production and has several industrial applications, including the manufacturing of polymer materials, soaps, lubricants, coatings, cosmetics, and pharmacological products, among others (Patel *et al.*, 2016). For these applications, castor oil is mainly valued for its high ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid) content, which typically accounts for 84 to 90% of its total fatty acid content (Fernández-Martínez and Velasco, 2012).

Castor is considered a crop with great potential for biorefinery (Naik *et al.*, 2018). However, little is known about the different compounds that will be valuable in biorefineries, such as tocopherols and phytosterols, in the castor germplasm (Granjo *et al.*, 2017). Tocopherols are antioxidants with both *in vivo* and *in vitro* free-radical scavenging capacities. While α-tocopherol shows powerful *in vivo* vitamin E activity (Azzi, 2018), other tocopherol forms, such as γ- and δ-tocopherol, exert strong *in vitro* antioxidant effects and are used as food and feed preservatives (Carocho *et al.*, 2018). Phytosterols are bioactive compounds which are widely used in cholesterol-lowering functional foods (Moreau *et al.*, 2018). Some of them, such as Δ^5-avenasterol are also powerful antioxidants with high-temperature applications (Rossell, 2001).

Information on the variability of seed and oil quality traits in the castor germplasm is scarce. Previous studies on the germplasm of the USDA-ARS collection (https://www.ars-grin.gov/npgs/index.html) have focused on evaluating hundred-seed weight, oil content, and fatty acid composition (Rojas-Barros *et al.*, 2004; Wang *et al.*, 2010; Wang *et al.,* 2011). Huang *et al.* (2015) evaluated the same traits in a collection of 32 accessions cultivated in China. Román-Figueroa *et al.* (2020) analyzed oil content and fatty acid profiles in 17 accessions collected in Chile. Velasco *et al.* (2015) reported the variability in hundred-seed weight, oil content, fatty acid profile, and tocopherol and phytosterol contents and profiles in a collection of 121 wild and semi-wild castor accessions from Spain. The latter authors identified substantial variability in all the traits evaluated, which indicated, for the first time, the existence of genetic diversity in compounds such as tocopherols and phytosterols in the castor germplasm.

The objective of the present research was to evaluate the genetic diversity in the abovementioned traits in the germplasm from the USDA-ARS collection, which includes cultivated accessions and covers more geographic diversity.

2. MATERIALS AND METHODS

2.1. Plant material and experimental design

One hundred and sixty accessions from the USDA-ARS germplasm collection were used for the study. Although the collection contains 1046 accessions (https://npgsweb.ars-grin.gov/gringlobal/search.aspx), the number of accessions used for the study was limited by the lack of availability of most of the accessions.

In May 2016, seeds from each accession were germinated at 25 °C in the dark on moistened filter paper discs placed in Petri dishes. After germination, 24 seeds per accession were sown in small pots of 7 × 7 × 8 cm and maintained in a growth chamber at 25 °C/20 °C (day/night) with a 16-h photoperiod for 3 weeks. After this time, the plants were transplanted into a field following a randomized block design with two replicates of 12 plants each. The plants were grown in single 4-m long rows with a 1-m separation between rows and a plant separation of 33 cm. The plants were irrigated periodically during the summer season. Before flowering, several racemes per plant were bagged using Kraft paper bags, as described by Fernández-Martínez and Velasco (2012). At maturity, the seeds from the bagged and open-pollinated racemes of each plant were collected. Each group of seeds (bagged and open-pollinated) was bulked per accession. In the case of plants with dehiscent capsules, the open-pollinated racemes were bagged at the end of flowering with transparent, microperforated plastic bags. Seed production in the summer of 2016 was discarded because many accessions produced no, or very few, fruits. Seeds for the study were thus obtained from three different environments, corresponding to three harvesting dates: November 2016, July 2017, and November 2017.

2.2. Seed analyses

The seeds were analysed for seed oil content using seeds from open-pollinated racemes and for fatty acid profile, tocopherol content and profile, and phytosterol content and profile using seeds from self-pollinated racemes. In all cases, the analyses were conducted in duplicate. Hundred-seed weight was also determined by counting and weighing duplicate samples of 100 seeds from the open-pollinated racemes.

The seed oil content was analyzed on intact, predried seeds at 103 °C for 17 hours, employing an Oxford 4000 nuclear magnetic resonance (NMR) analyzer from Oxford Analytical Instruments Ltd. in Abingdon, OX, UK.

For the analysis of fatty acids, tocopherols, and sterols, 12 individual half-seeds per accession were used in each case. Here, the term half-seed refers to a seed portion excised from the seed part distal to the embryo, approximately one-fifth of the seed length, so that the remaining seed part could be germinated to produce a new plant (Rojas-Barros *et al.*, 2004). This was done because, in some cases, seed production was low, and the seeds might be required for additional studies.

Fatty acid methyl esters were prepared by simultaneously extracting and methylating the seed samples following the procedure outlined by Rojas-Barros *et al.* (2004). Chromatographic analyses were conducted using a Perkin Elmer Clarus 600 GC (Perkin Elmer Inc, Waltham, MA, USA) equipped with a BPX70 30 m x 0.25 mm internal diameter x 0.25 µm film thickness capillary column (SGE Analytical Science Pty Ltd, Ringwood, Australia). The carrier gas was hydrogen, at a constant flow of 0.8 mL·min^{-1}. Split injector and flame ionization detector were set at 300 ºC. The initial oven temperature was set 140 ºC, held for two minutes, followed by a rate increase of 20 ºC·min^{-1} up to 250 ºC, held for 5 minutes.

For tocopherol analyses, the half seeds were finely crushed using a stainless-steel rod, and the resulting flour was weighed. Tocopherol extraction, separation by high-performance liquid chromatography (HPLC), and quantification were carried out in accordance with the methods described by Goffman *et al.* (1999), with a fluorescence detector with excitation at 295 nm and emission at 330 nm. An iso-octane/tert-butylmethylether (94:6) eluent was used at an isocratic flow rate of 0.8 ml·min^{-1}. Chromatographic separation of tocopherols was performed on a LiChrospher 100 diol column (250 mm x 3 mm internal diameter; Merck KGaA, Darmstadt, Germany) with 5-µm spherical particles, connected to a silica guard column (LiChrospher Si 60, 5 mm x 4 mm I.D.; Merck KGaA, Darmstadt, Germany). Rac-5,7-dimethyltocol (Matreya LLC, Pleasant Gap, PA, USA) was used as the internal standard for tocopherol quantification. Tocopherol standards (Calbiochem Tocopherol Set, Cat. No. 613424, Merck KGaA, Darmstadt, Germany) were used for the identification of the four tocopherols α-, β-, γ-, and δ-tocopherol. Total tocopherol content was calculated as the sum of the four tocopherols and expressed as mg per kg of seed kernel. The concentration of individual tocopherols was reported as a percentage of the total tocopherols.

Sterols were analyzed through gas-liquid chromatography (GLC) of the unsaponifiable fraction following silylation, without preliminary thin-layer chromatography (TLC) fractionation, as proposed by Fernández-Cuesta *et al.* (2012). This method permits the analysis of free and esterified desmethyl sterols directly on the seeds, without previous oil extraction. Gas chromatographic analyses were conducted using a Perkin Elmer Clarus 600 GC (Perkin Elmer Inc, Waltham, MA, USA) equipped with a ZB-5 capillary column with an internal diameter of 0.25 mm, a length of 30 meters, and a film thickness of 0.10 µm (Phenomenex, Torrance, CA, USA). Hydrogen served as the carrier gas at a flow of 0.8 mL·min^{-1}. The split injector and flame ionization detector were set at 320 °C. The oven temperature program began at 240 °C and increased at a rate of 5 °C per minute until reaching a final temperature of 265 °C, which was maintained for 10 minutes. 5α-cholestan-3β-ol (Cat. No. D6128, Merck KGaA, Darmstadt, Germany) was used as the internal standard. Phytosterol content was expressed as mg per kg of seed kernel. The concentration of individual phytosterols was reported as a percentage of the total phytosterols.

2.3. Statistical analyses

The results were subjected to analysis of variance using genotype and environment as fixed effects. Broad-sense heritability (H^2) was computed as the proportion of the sum of squares of the genotype in the analysis of variance (Steel and Torrie, 1980).

A comparison of the means was conducted using Tukey's post hoc test for multiple comparisons. Pearson's correlation coefficients between traits were computed using the averaged values of each accession across the three environments. The analyses were performed using IBM SPSS Statistics v 22 (IBM Corp., Armonk, NY, USA).

3. RESULTS

Table 1 shows the results of the analysis of variance conducted to estimate the relative effects of the genotype, the environment, and their interaction (G × E) on seed quality traits in the castor germplasm collection. The results revealed that the genotype, environment, and their interaction (G × E) were significant ($P < 0.01$) for all traits, except for the G × E interaction for the γ-tocopherol and δ-tocopherol concentrations. In the case of δ-tocopherol concentration, the interaction was significant at $P < 0.05$. The broad-sense heritability estimate was very high for the hundred-seed weight (H^2=0.88) and very low for the total tocopherol content (H^2=0.12). For the other traits, broad-sense heritability ranged from 0.21 for linoleic acid concentration to 0.52 for the concentration of γ-tocopherol (Table 1).

The mean values, standard deviation, and minimum and maximum values for all the traits evaluated in this study, averaged over the three environments for each accession, are presented in Table 2. Wide ranges of variation were observed for most traits. Hundred-seed weight averaged 26.0 g and ranged from 13.1 to 47.7 g. The average seed oil content was 51.8%, with a range of variation from 45.9 to 57.7 %. The fatty acid profile of the seed oil was mainly made up of 1.2% palmitic acid, 1.4% stearic acid, 4.3% oleic acid, 4.5% linoleic acid, and 87.5% ricinoleic acid. The major fatty acid in the seed oil was ricinoleic acid in all cases, which ranged from 83.1 to 89.7% of the total fatty acids.

The total seed tocopherol content averaged 176.9 mg·kg^{-1} in the collection, with a range of variation from 135.0 to 246.3 mg·kg^{-1} in the individual accessions. In all cases, γ-tocopherol was the main tocopherol derivative present in the seeds, accounting for 53.8 to 70.2% of the total tocopherols, followed by δ-tocopherol (22.7 to 41.8%) and α-tocopherol (3.4 to 9.2%) (Table 2).

The germplasm collection exhibited an average seed phytosterol content of 1246.0 mg·kg^{-1}, with a range of variation in individual accessions from

Table 1. Analysis of variance (sums of squares) and estimates of broad-sense heritability (H^2) for hundred-seed weight, seed oil content, concentration of major fatty acids in the seed oil, total tocopherol content, concentration of individual tocopherols, total phytosterol content, and concentration of major individual sterols in a collection of 160 castor accessions grown in three environments in Córdoba, Spain.

Trait	Genotype (%)	Environment	G × E	Error	H^2
Hundred-seed weight (g)	35983.9**	253.4**	4363.3**	280.7	0.88
Seed oil content (%)	4393.2**	396.1**	3050.0**	1078.8	0.49
Palmitic acid (% total fatty acids)	12.4**	2.0**	23.1**	17.2	0.23
Stearic acid (% total fatty acids)	38.1**	7.8**	47.4**	41.4	0.28
Oleic acid (% total fatty acids)	345.2**	112.1**	325.0**	323.4	0.31
Linoleic acid (% total fatty acids)	92.5**	5.1**	236.6**	106.3	0.21
Ricinoleic acid (% total fatty acids)	1010.8**	218.6**	1840.9**	966.8	0.25
Tocopherol content (mg kg^{-1})	383325.0**	2101848.4**	340260.7**	408424.8	0.12
α-Tocopherol (% tocopherols)	807.9**	634.2**	491.7**	586.8	0.32
γ-Tocopherol (% tocopherols)	8298.1**	34.0ns	5819.1ns	7659.8	0.52
δ-Tocopherol (% tocopherols)	10495.9**	492.1**	6767.4*	8631.7	0.40
Phytosterol content (mg kg^{-1})	13715257.8**	2639004.4**	17602558.8**	16377774.9	0.27
Campesterol (% phytosterols)	930.3**	297.5**	1380.7**	1104.5	0.25
Stigmasterol (% phytosterols)	2851.2**	606.6**	2665.8**	1629.8	0.37
β-Sitosterol (% phytosterols)	4778.8**	370.2**	5586.1**	3816.6	0.33
Δ^5-Avenasterol (% phytosterols)	4510.5**	311.4**	3286.4**	2297.0	0.43

*Significant at $p < 0.05$; **Significant at $p < 0.01$

Table 2. Mean, standard deviation (SD), minimum, and maximum mean values for hundred-seed weight, seed oil content, concentration of palmitic, stearic, oleic, linoleic, and ricinoleic acid in the seed oil, total tocopherol content, concentration of α-, γ-, and δ-tocopherol, total phytosterol content, and concentration of the individual sterols campesterol, stigmasterol, β-sitosterol, and Δ^5-avenasterol in a collection of 160 castor accessions grown in three environments in Córdoba, Spain.

Trait	Mean	SD	Minimum	Maximum
Hundred-seed weight (g)	26.0	6.2	13.1	47.7
Seed oil content (%)	51.8	2.2	45.9	57.7
Palmitic acid (% total fatty acids)	1.2	0.1	1.1	1.6
Stearic acid (% total fatty acids)	1.4	0.2	1.0	2.1
Oleic acid (% total fatty acids)	4.3	0.6	3.1	6.8
Linoleic acid (% total fatty acids)	4.5	0.3	3.9	6.2
Ricinoleic acid (% total fatty acids)	87.5	1.0	83.1	89.7
Tocopherol content ($mg \cdot kg^{-1}$)	176.9	20.4	135.0	246.3
α-Tocopherol (% tocopherols)	5.2	0.9	3.4	9.2
γ-Tocopherol (% tocopherols)	61.8	2.9	53.8	70.2
δ-Tocopherol (% tocopherols)	33.0	3.3	22.7	41.8
Phytosterol content ($mg \cdot kg^{-1}$)	1246.0	136.0	818.1	1589.4
Campesterol (% phytosterols)	8.4	1.3	5.3	14.4
Stigmasterol (% phytosterols)	21.1	2.1	13.0	28.4
β-Sitosterol (% phytosterols)	45.5	2.6	32.0	56.0
Δ^5-Avenasterol (% phytosterols)	18.8	2.5	10.7	26.3

818.2 to 1589.4 $mg \cdot kg^{-1}$ seed (Table 2). In all the accessions, β-sitosterol was the predominant sterol (32.0 to 56.0%), followed by stigmasterol (13.0 to 28.4%), Δ^5-avenasterol (10.7 to 26.3%), and campesterol (5.3 to 14.4%).

Table 3 shows the correlation coefficients between the main traits evaluated in this study. Hundred-seed weight showed a positive significant ($P < 0.01$) correlation with both oil content (r=0.29) and ricinoleic acid concentration (r=0.33), and a negative correlation with total tocopherol content (r=-0.37). Oil content was also positively correlated with total phytosterol content (r=0.20). Total tocopherol content was positively correlated with the percentage of δ-tocopherol (r=0.33) and negatively correlated with the percentage of γ-tocopherol (r=-0.29). Both tocopher-

Table 3. Correlation coefficients between hundred-seed weight (g), seed oil content (%), concentration of ricinoleic acid in the seed oil (% total fatty acids), total tocopherol content (mg kg^{-1} seed), concentration of major tocopherols (% tocopherols), total phytosterol content (mg kg^{-1} seed), and concentration of major individual sterols (% phytosterols) in a collection of 160 castor accessions grown in three environments in Córdoba. Spain.

	Oil	Ricinoleic	Tocopherol	γ-Tocopherol	δ-Tocopherol	Phytosterol	Stigmasterol	β-Sitosterol	Δ^5-Avenasterol
Hundred-seed weight	0.29**	0.33**	-0.37**	0.16*	-0.19*	0.17*	-0.13	-020*	0.16*
Oil		0.03	0.03	0.09	-0.10	0.20**	-0.17*	-0.02	0.13
Ricinoleic			-0.17*	-0.11	0.05	-0.11	0.12	-0.28**	0.06
Tocopherol				-0.29**	0.33**	-0.02	0.18*	0.02	-0.09
γ-Tocopherol					-0.96**	0.22**	-0.11	0.12	-0.03
δ-Tocopherol						-0.24**	0.07	-0.15	0.13
Phytosterol							-0.38**	0.44**	0.06
Stigmasterol								-0.18*	-0.46**
β-Sitosterol									-0.35**

*Significant at $p < 0.05$; **Significant at $p < 0.01$

ol derivatives showed a strong negative correlation (r=-0.96). Phytosterol content was positively correlated with the concentration of β-sitosterol (r=0.44) and negatively correlated with the concentration of stigmasterol (r=-0.38). Δ^5-avenasterol concentration was negatively correlated with the concentration of both stigmasterol (r=-0.46) and β-sitosterol (r=-0.35). Other significant correlation coefficients ($P < 0.01$) were observed between the concentrations of ricinoleic acid and β-sitosterol (r=-0.28) and between phytosterol content with the concentrations of both γ-tocopherol (r=0.22) and δ-tocopherol (r=-0.24). Other correlation coefficients with lower statistical significance ($P < 0.05$) are also presented in Table 3.

4. DISCUSSION

Broad-sense heritability (H^2) indicates the proportion of phenotypic variance that is attributable to the genotype and, accordingly, anticipates the response to selection for a given trait (Schmidt *et al.*, 2019). In this study, we identified a very high heritability estimate for the hundred-seed weight (H^2=0.88), very low for the total tocopherol content (H^2=0.12), and intermediate for other traits (H^2 from 0.21 to 0.52). Velasco *et al.* (2015) reported a similar heritability estimate for hundred-seed weight, 0.90, in the evaluation of a collection of wild and semi-wild landraces collected in Spain. The authors also reported high estimates for Δ^5-avenasterol (0.85) and β-sitosterol content (0.79), which were much higher than the values observed in the present research. The reason for these differences was the identification of a set of accessions from a specific location with an unusually high content of Δ^5-avenasterol (> 40%), probably caused by a gene mutation (Velasco *et al.*, 2015). The authors also reported a much higher genotypic contribution to the variation in the total tocopherol content (0.66). The substantial difference between this result and that in the present study may also lie in the identification by Velasco *et al.* (2015) of accessions with high or low levels of tocopherols, which were expressed in two environments, as well as in the originally collected seeds (Velasco *et al.*, 2015).

The range of variation for hundred-seed weight found in the present study (13.1 to 47.7 g) was similar to that reported by Huang *et al.* (2015) in accessions collected in China (10.9 to 45.2 g) and smaller than that reported by Wang *et al.* (2010), who evaluated a higher number of accessions from the USDA germplasm collection and found that this trait varied between 10.1 and 73.3 g. It is important to note that the study of Wang *et al.* (2010) was based on seeds from the gene bank, whereas the present research reports average values from plants grown in three environments. Broader variability, from 11.6 to 59.1 g, was also reported by Velasco *et al.* (2015) in the evaluation of a collection of Spanish wild and semi-wild landraces in two locations.

Seed oil content varied from 45.9 to 57.7%, which is close to the ranges of variation reported by Rojas-Barros *et al.* (2004) in the analysis of 191 accessions from the USDA germplasm collection grown in a single environment (44.8 to 56.5%), Velasco *et al.* (2015) in the Spanish collection of wild and semi-wild populations, and Román-Figueroa *et al.* (2020) in accessions from Chile (45.7 to 54.0%). In the analysis of the whole USDA world collection, Wang *et al.* (2010) reported a broader range of variation for this trait (37.2 to 60.6%), although it is important to note that the authors analyzed the seeds that were acquired from the gene bank. Huang *et al.* (2015) found lower levels of oil content in accessions cultivated in China (36.6 to 49.2%).

The fatty acid profile of the accessions analyzed in this study was similar to those reported in previous studies on castor germplasm variability (Rojas-Barros *et al.*, 2004; Wang *et al.*, 2011; Velasco *et al.*, 2015; Huang *et al.*, 2015; Román-Figueroa *et al.*, 2020). The variability in the ricinoleic acid concentration was slightly higher in Wang *et al.* (2011) (78.3 to 88.0%) and Rojas-Barros *et al.* (2004) (79.4 to 87.6%). In the former case, the reason might be the analysis of gene bank seeds compared to the analysis of seeds from three environments in the present research. In the latter case, the authors identified one accession containing seeds with high oleic acid and, subsequently, a low ricinoleic acid concentration.

The variation in total tocopherol content (from 135.0 to 246.3 $mg \cdot kg^{-1}$ seed) was lower than that reported previously in the Spanish germplasm collection of wild and semi-wild accessions (99.6 to 332.0 $mg \cdot kg^{-1}$ seed) (Velasco *et al.*, 2015). As stated above, the mentioned authors identified accessions with low or high tocopherol levels, which resulted in higher heritability and a broader range of variation than in the present research. The mean values for the

tocopherol profile were 5.2% α-tocopherol, 61.8% γ-tocopherol, and 33.0% δ-tocopherol Although these values are very similar to those reported by Velasco *et al.* (2015), the variability was higher in the mentioned study. For example, Velasco *et al.* (2015) reported variation between 27.4 and 50.5% in δ-tocopherol concentration whereas levels of 22.7 to 41.8% were found in the present research. No other studies on tocopherol content or profile of the castor germplasm have been conducted thus far.

The phytosterol content in the accessions included in our research exhibited a narrower range of variation (818.2 and 1589.4 $mg \cdot kg^{-1}$) than that identified in the Spanish germplasm collection of wild and semi-wild accessions (1090.5 to 2865.5 $mg \cdot kg^{-1}$). The difference was mainly caused by the presence of one accession with a very high phytosterol content in the Spanish collection (Velasco *et al.*, 2015). These authors also reported an average phytosterol profile with a higher Δ^5-avenasterol percentage (25.2%, compared to 18.8% in the present study) and concomitantly lower concentrations of the other sterols. This was mainly caused by the presence of a group of accessions with a very high Δ^5-avenasterol content of up to 54.1%, much higher than the highest value (26.3%) found in the USDA collection.

Some of the significant correlation coefficients observed in this research have also been reported in previous studies. Thus, a positive correlation between hundred-seed weight and oil content was previously reported by Wang *et al.* (2011) and Velasco *et al.* (2015). In addition, the latter authors found that hundred-seed weight was positively correlated with ricinoleic acid concentration and negatively correlated with tocopherol content, which was also observed in the present study. Correlations between hundred-seed weight and tocopherol profile, previously reported by Velasco *et al.* (2015), were also observed in the present research, although with a lower level of significance ($P < 0.05$). The strong negative correlation between oil and tocopherol content reported by these authors was not observed in this germplasm collection. The positive correlation of total tocopherol content with the concentration of δ-tocopherol and the concomitant negative correlation with the concentration of γ-tocopherol were also reported by Velasco *et al.* (2015). Negative correlation coefficients were found among the three main sterols in castor seeds, stigmasterol, β-sitosterol, and Δ^5-avenasterol. This was observed by Velasco *et al.* (2015) as well, except for the correlation between stigmasterol and β-sitosterol, which was significant at $P < 0.05$ in the present study. The negative correlations between the three sterols are explained based on their common biosynthetic pathway, where stigmasterol is synthesized from β-sitosterol, and this is in turn synthesized from Δ^5-avenasterol (Nes, 2011).

A previous study evaluated the variability in seed weight, seed oil content, fatty acid profile, and tocopherol and phytosterol contents and profile of a germplasm collection of wild and semi-wild accessions collected in Spain (Velasco *et al.*, 2015). For accessions from a broader geographical area, including cultivated accessions, Wang *et al.* (2010); Wang *et al.* (2011) and Rojas-Barros *et al.* (2004) reported information on seed weight, oil content, and fatty acid profile. Those studies were based on the analysis of seeds from the gene bank (Wang *et al.*, 2010 and Wang *et al*., 2011) or from plants grown in a single environment (Rojas-Barros *et al.*, 2004). The present research provides, for the first time, valuable information on all these traits in a germplasm collection containing accessions with substantial geographic diversity, based on the analysis of seeds collected from three environments. The higher variability identified in previous studies was probably caused by environmental effects in the case of the analysis of gene bank seeds (Wang *et al.*, 2010 and Wang *et al.,* 2011), or by the wild or semi-wild nature of the accessions (Velasco *et al.*, 2015). Wild relatives contain greater genetic diversity than cultivated forms owing to population bottlenecks during the domestication process (Liu and Burke, 2006).

Biorefineries offer better economic possibilities and a wider market for castor crops than the mere exploitation of seed oil for either biofuel or industrial applications (Dimian *et al.*, 2019). Knowledge about the variability in the castor germplasm of the industry's bioactive compounds of interest is essential for developing cultivars that contribute to maximizing the profitability of biorefineries. Castor seeds contain powerful antioxidants such as γ-tocopherol, δ-tocopherol (Carocho *et al.*, 2018), and Δ^5-avenasterol (Rossell, 2001), as well as other sterols with applications as bioactive compounds with cholesterol-lowering properties (Moreau *et al.*, 2018). The present research provided, for the first time, insight into the variability of these compounds in a ger-

mplasm collection of castor accessions, including cultivated accessions, from a broad geographic distribution. The variation in seed quality traits reported in the present research can be exploited in breeding programs aimed at improving the concentration of bioactive compounds in castor seeds.

5. CONCLUSIONS

Castor accessions from a broad geographical origin contain great genetic variability for bioactive compounds such as tocopherols and phytosterols. Such variability is lower than that reported previously for wild and semi-wild accessions of the species, which is a common observation in cultivated vs. wild species. Knowledge about the existing variability in castor germplasm and the relative influence of phenotypic and environmental factors will be of great value for defining future uses for castor oil and optimized breeding strategies for this promising oilseed crop.

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7. DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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